REMARKS

Claims 109-111, 116, and 119-127 are pending. Claims 1-108 and 118 are cancelled. Claims 112-115, 117, and 128-130 have been withdrawn. Claims 109 and 127 have been amended. Support for the amendments may be found, in part, at paragraph [0056], paragraph [0212], and figures 13 and 29. Claims 131-142 are new. Support for the new claims may be found in figures 13 and 29.

A supplementary IDS, citing the Tanaka et al. chapter referenced in section III, has been filed herewith.

I. Summary of August 20, 2009 telephone conference

Applicants would like to thank Examiner Bhat for the constructive telephone interview on August 20, 2009. The Baez reference was discussed and it was mentioned that free energy calculations based on the temperature and salt ranges disclosed in Baez are outside the scope of claim 109, especially as presently amended. An overview of the free energy calculations are presented in Section II below. As a result, the Baez reference does not anticipate claim 109.

In further discussions, Examiner Bhat stated that the Baez reference, while not anticipatory, rendered claim 109 obvious. Applicant's disagree, and Section III below addresses the obviousness issue.

II. Baez et al. Does Not Anticipate the Biosensor of Claim 109

Reconsideration is requested of the rejection of claims 109-111, 116, 119-122 and 124-127 under 35 USC §102(b) for anticipation in light of Baez et al., as evidenced by the HYTHER program.

For Baez to anticipate claim 109, Baez would have to:

(A) disclose a reporter with each element of claim 109, and

(B) disclose the arrangement of each element as required by claim 109.[1]

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[1] "Anticipation under § 102 can be found only when the reference discloses ***exactly*** what is claimed . . ." Titanium Metals Corp. v. Banner, 778 F.2d 775, 780 (Fed. Cir. 1985) (citing D.

As detailed below, the Baez application fails to do either. Consequently, Baez does not anticipate claim 109.

(a) the Baez reporter does not disclose each element of claim 109

Amended claim 109 is directed to a biosensor comprising two constructs (R1-R2-R3-R4 and R5-R6-R7-R8). Claim 109 requires that R3 is a complementary nucleotide sequence having a free energy for association, over the entire length of the nucleotide sequence, from about 5.5 kcal/mole to 8.0 kcal/mole. The Baez application fails to disclose an equivalent to R3.

Baez discloses a complementary nucleic acid sequence of CGCCCGA. Using the hybridization conditions taught in Baez (25°C and KCl between 20-60mM)[2], the Baez sequence does **NOT** have a free energy range between about 5.5 kcal/mol and 8.0 kcal/mol, as required by Claim 109.

The Office has previously asserted that the Baez sequence has a free energy of 6.05 kcal/mol at 37°C and 50mM salt.[3] This, however, is the ***wrong temperature*** to evaluate the Baez sequence. The Baez application explicitly states:

> "[a] 7 to 10 bp overlap of the 3' ends of the labels, which has an approximate T_m of 25°C, was used to ***avoid formation of the duplex at 37°C***. (the incubation temperature used for antibody-analyte binding)…. However, after the analyte-reporter complex is formed and ***the temperature reduced to 25°C, the 3' overlap is allowed to form***."[4]

Chisum, Patents § 3.02). "Because the hallmark of anticipation is prior invention, the prior art reference – in order to anticipate under 35 U.S.C. § 102 – must not only disclose all elements of the claim within the four corners of the document, ***but must also disclose those elements "arranged as in the claim."*** Net Moneyin, Inc. v. Verisign, Inc., 545 F.3d 1359, 1369 (Fed. Cir. 2008) (citing Connell v. Sears, Roebuck & Co., 722 F.2d 1542, 1548 (Fed. Cir. 1983). Baez does not disclose the R2/R6 and R3/R7 elements arranged as required by claim 109 of the present application. Consequently, Baez does not anticipate claim 109.

[2] See paragraph [0131] of the Baez application.

[3] It is noted that the 6.05 kcal/mol value asserted by the Office was actually obtained using the sequence CGCCGA and not the correct sequence CGCCCGA, according to the HYTHER conditions appended to the March 12, 2009 Office Action.

[4] See Baez application at paragraph [0184].

Hence, the temperature for evaluating the free energy of the Baez sequence is 25°C, not 37°C.

In the Advisory Action mailed June 23, 2009, the Office again asserts that paragraph 0225 of Baez teaches a temperature for 3' overlap formation between 25°C and 45°C. This is not accurate. Paragraph 0225 specifically states: "temperatures for incubation could range within the permissible temperature tolerance of the **antigen-antibody interaction**, for example between 25°C and 45°C." The temperature range detailed in paragraph 0225, therefore, is for the antigen-antibody interaction, and **NOT** for the 3' overlap. As stated above, Baez explicitly teaches that the 3' overlap is designed to avoid formation of the duplex at the incubation temperature used for antibody-analyte binding. Hence, the appropriate temperature for evaluating the free energy of the Baez sequence is 25°C, not 37°C.

The free energy of the Baez sequence, at 25°C and over the range of salt conditions detailed in the Baez application (i.e. 20mM to 60mM)[5], **is outside the range of claim 109** (see **Table A**). The Office has asserted in the Advisory Action that a free energy of 8.65, as detailed in Table A, is within "*about* 8.0kcal/mol." Applicants disagree, but to expedite prosecution, have amended claim 109 to remove the "about." As a result, the required free energy range of claim 109 is about 5.5 kcal/mol to 8.0 kcal/mol, and 8.65 kcal/mol lies outside of that range. Hence, the Baez application **DOES NOT** disclose the equivalent to R3, and consequently, **DOES NOT** anticipate claim 109.

Table A

Temperature (°C)	**Salt**	**Free Energy**
25	20mM	8.65
25	50mM	9.25

[5] See paragraph [0131] of the Baez application.

25	60mM	9.37

(b) Baez does not disclose the arrangement of each element as required by claim 109

Even if Baez disclosed an equivalent to R3 of claim 109, Baez does not disclose the arrangement of each element as required by claim 109. In particular, claim 109 requires that R2, a non-nucleic acid linker, is attached to a complementary nucleotide sequence having a free energy for association, over the entire length of the nucleotide sequence, from about 5.5 kcal/mole to 8.0 kcal/mole. Baez does not disclose this arrangement of claim elements.

Depending on which of the Office's interpretations of the Baez reference is examined, Baez either (A) discloses a linker comprised of a heterobifunctional moiety and nucleic acid[6], which falls outside of the limitations of R2, **OR**, (B) Baez discloses a heterobifunctional moiety attached to a nucleic acid sequence that comprises both non-complementary and complementary nucleic acid sequence[7] that falls outside of the free energy limitations of R3. Either interpretation fails to meet the required elements of claim 109, as detailed in **Table B** below.

Table B

Claim 109	**Baez disclosure, interpreted by the Office in the July 2, 2008 Office Action**	**Baez disclosure, interpreted by the Office in the March 12, 2009 Office Action**
R1 / R5 epitope binding agent	Antibody	Antibody

[6] The interpretation put forth by the Office in the July 2, 2008 office action.
[7] The interpretation put forth by the Office in the March 12, 2009 office action.

R2 / R6 non-nucleic acid linker attaching R1 to R3	Heterobifunctional moiety and non-complementary ***nucleic acid***	Heterobifunctional moiety
R3 / R7 • complementary nucleic acid sequences • **free energy between about 5.5 and 8.0 kcal/mol over the entire length of the sequence** • temperature about 21°C to 40°C • salt at about 1mM to 100mM	complementary nucleic acid sequences that falls outside the free energy requirements of claim 109 (see Table A above)	***non-complementary*** and complementary nucleic acid sequence that falls outside the free energy requirements of claim 109 (see Table C below)
R4 / R8 Detection means	Detection means based on [00139] of the Baez application	Detection means based on [00139] of the Baez application

If the Office's March 12, 2009 interpretation is used, then the Baez equivalent of R3 comprises non-complementary and complementary nucleic acid sequence. Claim 109 requires that R3 is a complementary nucleotide sequence having a free energy for association [with R7], **over the entire length of the nucleotide sequence**, from about 5.5 kcal/mole to 8.0 kcal/mole. The mixed complementary and non-complementary sequence of Baez, however, **fails** to meet the free energy requirements of claim 109, regardless of the conditions used.

Table C details the free energies of the nucleic acid portion of Baez (as calculated by the Hyther program)[8] under the hybridization conditions detailed in Baez (i.e. 25°C and between 20 and 60mM NaCl)[9]. The corresponding free energy range is between 26.98 and 31.33, which is outside the scope of claim 109. Additionally, Table C details the free energy of the Baez nucleic acid portion under the high and low temperatures (about 21°C to about 40°C) of claim 109 along with a range of salt concentrations claimed (10mM to 100mM)[10]. As evidenced by the values in the Table (the lowest of which is 22.96 kcal/mol), this interpretation of the nucleic acid portion of the Baez reporter falls outside of the free energy requirement of claim 109 that recites " from about 5.5 kcal/mol to 8.0 kcal/mol." As a result, the Baez reference cannot anticipate claim 109.

Table C

Temperature (°C)	**Salt**	**Free Energy**
25	20mM	31.33
25	60mM	26.98
21	100mM	22.96
40	100mM	32.47
21	10mM	31.95
40	10mM	42.04

The Office has previously argued that the "comprising" language in claim 109 allows the insertion of random nucleic acid between the linker (R2) and R3. This is not the case. Claim 109 requires that R2 is a *non-nucleic acid flexible linker* ***attaching R1 to R3***. R2 must be non-nucleic acid, per claim 109. R3 has to meet the free energy standards stated in claim 109. Hence, non-

[8] For a summary of the HYTHER calculation conditions, see Appendix 1.
[9] See paragraph [0131] of the Baez application. The annealing temperature was derived from the table following paragraph [0131] and Examples 1 and 2.
[10] Claim 109 is restricted to about 1mM to about 100mM. The HYTHER program, however, will not calculate free energy for salt conditions below 10mM. As a result, 10 mM is used in the calculations herein to represent the lower side of the salt concentration range.

complementary sequence between R2 and R3 is only permissible **IF** it meets the free energy requirements of R3. (As R2 must connect R1 to R3 and R2 can't be nucleic acid. Otherwise, R2 is connecting R1 to the random nucleotide sequence, not R3.) And, as stated above, the nucleic acid portion of the Baez reporter **DOES NOT** meet these requirements (see **Table C**).

Similar to claim 109, claims 110, 111, 116, 119 –122, and 124 -126 each depend from claim 109, and therefore necessarily incorporate each limitation of claim 109. Consequently, the Baez application cannot anticipate claims 110, 111, 116, 119 –122, and 124 -126 for the same reasons as detailed above with respect to claim 109.

Claim 127, analogous to claim 109, requires that R2 and R6 are not comprised of nucleic acid, and that R3 and R7 have a free energy of association, over the entire length of the nucleotide sequence, between about 5.5 kcal/mol and 8.0 kcal/mol. The Baez application, which only discloses linkers of nucleic acids and does not disclose a complementary nucleic acid region with a free energy between about 5.5 kcal/mol and 8.0 kcal/mol, does not, therefore, anticipate claim 127 for the same reasons detailed above with respect to claim 109.

New claim 131 is directed to a biosensor consisting of R1-R2-R3-R4 and R5-R6-R7-R8. Because claim 131 recites "consisting of," as a matter of law, the biosensor can only comprise the elements R1-R2-R3-R4 and R5-R6-R7-R8. Hence, Baez does not anticipate claim 131 because the non-complementary sequence of Baez is not encompassed by the elements R2 or R3 (see Table B above). Additionally, the Baez application does not anticipate claim 131 for the same reasons as detailed above with respect to claim 109. New claims 132-142 depend from claim 131 and necessarily incorporate each limitation of claim 131. As a result, the Baez application does not anticipate claims 132-142 for the same reasons as detailed above with respect to claim 131 and 109.

Consequently, Applicant requests withdrawal of the rejection of claims 109-111, 116, 118-122 and 124-127 under §102b in view of Baez et al.

III. Baez et al. Does Not Render the Biosensor of Claim 109 Obvious

Reconsideration is requested of the rejection of claims 109 and 123 under 35 USC §103(a) in view of Baez et al, as evidenced by the HYTHER program and Zalipsky.

(a) importance and unexpectedness of the free energy range of claim 109

The biosensor of the invention is designed to produce a signal in the presence of a particular target. The epitope binding agents (i.e. R1 and R5) bind to the target. This brings R3 and R7 into close proximity, and the flexible linkers R2 and R6 allow R3 and R7 to form a duplex DNA sequence. The formation of the duplex brings the two labels, R4 and R8 into proximity and produces a signal.

The system has a carefully crafted internal balance: if R3 and R7 bind too tightly, they will bind in the absence of target, creating high background signal. Alternatively, if they do not bind tightly enough, the signal in the presence of target will not be robust. The free energy range detailed in claim 109 addresses this internal balance, and is central to the claimed biosensor.

To demonstrate the importance of the free energy, Applicants direct the Office's attention to the second attached declaration by Dr. Heyduk. The declaration clarifies and re-iterates an experiment originally detailed in the declaration filed 2/30/08. The experiment was designed to detect C-peptide with two different biosensors, and was performed at 25°C and at a NaCl concentration of 100mM, which is within the parameters of claim 109.[11] The ***only*** difference between the two biosensors used in the experiment is the free energy range of

[11] Claim 109 requires a temperature from about 21°C to about 40°C and a salt concentration from about 1mM to about 100mM. The Office has stated that the experiment detailed in the declaration is not commensurate with the scope of claim 109. This is incorrect. The experiment is performed at a NaCl concentration of 100 mM, which is within the range of 1M to 100mM cited in claim 109. The experiment was also performed at 25°C, which is within the range of 21°C to 40°C required by claim 109. The Office seems to have mistaken the calculations of free energy in part 2(b) of the declaration to be the conditions of the experiment in part 2(a).

the R3/R7 components of the biosensor. One biosensor uses the Baez sequence, which, under these conditions, has a free energy outside the scope of claim 109. The other sensor uses a sequence (ATGAGC) that does have a free energy range within the scope of claim 109. As Dr. Heyduk noted:

> Robust signal was obtained **only** with the biosensor that comprised an R3/R7 pair that has a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol, as required by claim 109.

Stated another way, the free energy of the R3/R7 biosensor determined whether a signal was produced or not. **This importance of the free energy range to the function of the sensor was not previously known and was unexpected**.

(b) there is no prima facie case of obviousness

Three criteria must be present to establish a *prima facie* case of obviousness.[12] First, the prior art reference must teach or suggest *all* the claim limitations. Second, there must be some suggestion or motivation in the knowledge generally available to one of ordinary skill in the art to modify the reference. Third, there must be a reasonable expectation of success.[13] Not one of these three criteria is satisfied by the combination of the Baez application, the HYTHER program, and the Zalipsky reference.

i. the cited references do not disclose all the limitations of claim 109

As discussed above, the Baez application does not disclose an equivalent to the R2-R3 portion of claim 109. Importantly, the Baez application does not disclose a complementary nucleic acid region with a free energy within the requirements of claim 109.

Resorting to the Zalipsky reference does not cure the defect in the Office's obviousness rejection. The Zalipsky reference discloses polyethyleneglycol

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[12] MPEP §2143
[13] *Id.*

(PEG) as a *conjugate* for biologically active molecules. By attaching PEG to the biologically active molecule, the molecule is stabilized. The Zalipsky reference **DOES NOT** disclose using PEG as a ***linker*** between ***two*** agents (e.g. an epitope binding agent and a complementary nucleic acid in a biosensor). Stated another way, the Zalipsky reference **does not** disclose attaching a chain of PEG to an epitope binding agent to connect the agent to a complementary nucleic acid. Nor, for that matter, does Zalipsky teach or suggest, in any manner, connecting ***two*** biomolecules with PEG.

In summary, ***not one of the cited references, whether taken individually or in combination, disclose a biosensor of claim 109***.

ii. there is NO suggestion, motivation, or reasonable expectation of success to modify the references

There is no suggestion or motivation in the art or the references provided by the Office to modify the Baez reference to arrive at the biosensor of claim 109.

1. There is no suggestion, motivation, or reasonable expectation of success to modify the Baez reference so as to satisfy the free energy requirements of claim 109.

As detailed in section I above, the Baez reference does not disclose the R2-R3 portion of claim 109. Additionally, the Baez reference provides no motivation or suggestion to alter the Baez reporter to satisfy the requirements of claim 109. In particular, there is no motivation to use a flexible non-nucleic acid linker (R2) attached to a nucleic acid sequence that has a free energy for association, over its entire length, of between about 5.5 kcal/mol and 8.0 kcal/mol (R3).

In fact, the Baez reference ***teaches away*** from such an arrangement of elements. For instance, the Baez reference teaches a non-nucleic acid heterobifunctional moiety (which the Office refers to as R2) attached to a non-complementary nucleic acid sequence. The nucleic acid sequence does not have

a free energy within the limitations of claim 109. The Baez reference specifically teaches including the non-complementary sequence in the reporter, stating that the "unhybridized nucleic acid labels are flexible."[14] Hence, by teaching the inclusion of a non-complementary sequence, the Baez reference teaches away from a sequence that has a free energy, over its entire length, within the confines of claim 109.

The Office has argued that it would be known in the art to modify the reaction conditions to arrive at the free energy of claim 109. In other words, that it would have been routine optimization to modify the free energy of the Baez reporter to arrive at the requirements of claim 109. This argument is erroneous and misses the point. While it is known in the art that modifying reaction conditions will effect free energy, **it was NOT known in the art that the free energy range in claim 109 produced the increased sensitivity and specificity observed with the biosensor of claim 109.**

The Office's argument of routine optimization is without basis. A particular parameter must first be recognized as a result-effective variable, *i.e.*, a variable which achieves a recognized result, before the determination of the optimum workable ranges of said variable might be characterized as routine experimentation. Before the applicants' patent application, it would have been unexpected that the currently claimed free energy range would affect the signal produced by the biosensor to the drastic extent illustrated in Dr. Heyduk's declaration.

Before the Office can summarily dismiss a claim element as being a parameter that can be "routinely optimized" the burden is on the Examiner to establish that the given parameter is **a result-effective variable.** Only result-effective variables can be optimized. As specifically dictated by MPEP § 2144.05:

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[14] Baez at paragraph [0113].

> …A particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum workable ranges of said variable might be characterized as routine experimentation.

Against the backdrop of this legal standard, the Office hasn't established that the claim element relating to the free energy of the R3/R7 portion of the biosensor is "**a result-effective variable.**" Nowhere does Baez disclose or suggest any impact of the free energy on the ability of biosensor to produce a signal. In fact, Baez is **completely silent** regarding any correlation between free energy and biosensor performance.

In contrast, the applicants have demonstrated the critical nature of the claimed range for free energy and the unpredictable results derived from this claimed range. As previously stated, the applicants have demonstrated that **only** R3/R7 sequences with free energy values, over the entire nucleic acid, of about 5.5kcal/mol and 8.0 kcal/mol produces a signal within the conditions required by claim 109. As stated above and in the declaration by Dr. Heyduk, if a nucleic acid sequence with a free energy outside of the scope of claim 109 is used, there is **no signal** produced by the biosensor. As such, the claimed free energy range is both **a critical and unpredictable range**.

Nowhere does Baez disclose or suggest the importance of this range. Instead, Baez merely teaches that the nucleotide composition and length of the sequence may influence the temperature range of hybridization. These parameters are not equivalent to free energy. In 2006, a year after the priority date of the present application, Tanaka et al., in their book chapter entitled "Specificity of Hybridization Between DNA Sequences Based on Free Energy," state that it was **not known** whether sequence design with the appropriate hybridization specificity is best based on the nucleic acid composition or on the free energy.[15] Hence, **IT WAS NOT KNOWN IN THE ART AT THE TIME OF**

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[15] DNA Computing: Specificity of Hybridization Between DNA Sequences Based on Free Energy (2006) pages 371-379, at page 371.

FILING that free energy was a result effective variable for nucleic acid hybridization.[16]

In view of this, the recited free energy element of claim 109 is very clearly not a result effective variable. In fact, it is the very opposite of a result effective variable. The Office cannot, therefore, state that the free energy range required by claim 109 would have been "routine optimization" of the Baez reporter.

2. There is no suggestion, motivation, or reasonable expectation of success to modify the Baez reference in light of the Zalipsky reference.

Additionally, there is no suggestion, motivation, or reasonable expectation of success in the art to modify the Baez reporter with a PEG linker. The Zalipsky reference **DOES NOT** teach or suggest using PEG as a *linker between **two** biomolecules*. Instead, Zalipsky, as described above, discloses merely conjugating PEG to a biomolecule (i.e. connecting PEG to a ***single*** biomolecule, as opposed to linking ***two*** biomolecules with PEG). Zalipsky does not teach or suggest that PEG may be used for linking two biomolecules, as required by the biosensor of claim 109.

The Office asserts that "an artisan would have been motivated to use the PEG linker in the sensor of Baez with the expected benefit of increasing stability and solubility of the polyethylene glycol conjugate as taught by Zalipsky." This conclusion, however, is inappropriately equating the use of PEG as a conjugate (as disclosed in Zalipsky) to the use of PEG as a linker (which is **not** disclosed in Zalipsky). Furthermore, to use PEG as a linker in the biosensor of claim 1, the PEG must be attached to a nucleic acid (R3). Nowhere does Zalipsky teach the attachment of PEG to a nucleic acid. Hence, there is no suggestion or motivation to use the PEG of Zalipsky as a ***linker*** in the Baez reporter.

Without a demonstration of the requisite motivation to make the Office's proposed modification, a *prima facie* case of obvious has not been established.

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[16] The authors did conclude, however, that free energy "is superior" to nucleic acid composition "in terms of the capability to separate specific from non-specific sequences."

Moreover, the Office has not established that a skilled artisan would have a reasonable expectation of success if its proposed modification were made.

In light of the foregoing, Applicants respectfully request withdrawal of the rejection of claims 109 and 123 under 35 USC §103 for obviousness.

Additionally, new claims 131-142 are not rendered obvious for the same reasons as detailed above with respect to claim 109.

CONCLUSION

In light of the foregoing, applicants request entry of the claim amendments, withdrawal of the claim rejections, and solicit an allowance of the claims. The Examiner is invited to contact the undersigned attorney should any issues remain unresolved.

Respectfully submitted,

Polsinelli Shughart PC

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